Current Options and New Developments in Hemophilia

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Disclosures

• Research support to OHSU from:
  – Baxter
  – Pfizer
  – NovoNordisk

Agenda

• Early treatment of hemophilia
• Factor repletion
• Treatment of patients with inhibitors
• Adjuvant therapies
• Future directions
  – Gene therapy
  – Premature termination codon suppression
Abbreviations used

- FVIII = factor VIII
- recFVIII = recombinant factor VIII
- pdFVIII = plasma-derived factor VIII
- FIX = factor IX
- recFIX = recombinant factor IX
- pdFIX = plasma-derived factor IX
- FVIIa = activated factor VII
- recFVIIa = recombinant activated factor VII

History of hemophilia therapy

- Prior to the 1940’s-supportive care and whole blood transfusion
  - Low concentration of factors VIII and IX
  - Individuals suffered significant pain and morbidity
  - Average lifespan 27 years
- 1964-Judith Graham Pool described method to produce cryoprecipitate
  - Rich in FVIII and fibrinogen
  - Beginning of home infusions
  - Average lifespan 40 years

History of hemophilia therapy

- 1970's-plasma-derived factor concentrates
  - Pools of 20,000+ donors
  - Made school, work, and travel possible
  - Average lifespan 60 years
However….

• Hepatitis B and C were known to be in plasma supply
• Thought to be an “acceptable” risk in light of drastic improvement in quality of life
• First individual with hemophilia that died from HIV infection reported in 1982
  – Only retrospectively was it discovered that plasma-derived factor was vector
  – HIV was isolated in 1984
  – Heat treatment of plasma-derived factor became standard practice in 1985

Recombinant factor VIII

• FVIII gene sequenced and cloned in 1984
• Because of complex glycosylation and post-translational modification of FVIII protein, recombinant FVIII can only be produced in mammalian cell culture
• First clinical trial of recombinant FVIII concentrate reported in 1990
• First recombinant FVIII concentrate marketed for clinical use in 1992

Recombinant FVIII generations

• First generation
  – Required bovine or human albumin to stabilize FVIII molecule
• Second generation
  – Albumin required during manufacturing process, but removed from final product
• Third generation
  – Albumin free during entire manufacturing process
  – Purification process removes impurities from medium and cell culture
Current FDA-approved recombinant FVIII concentrates

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<th>Product</th>
<th>Manufacturer</th>
<th>Viral inactivation steps</th>
<th>Stabilizer</th>
<th>Proteins in product</th>
<th>Viral safety studies</th>
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Viruses in recFVIII concentrates

- Viral transmission has never been reported in any recombinant FVIII product
- Theoretical risk exists in first and second generation products
- Non-viral pathogens such as prions possible in first and second generation products

Recombinant FIX

- Human FIX gene was cloned in 1982
- Minor differences in post-translational sulfation between recFIX and pdFIX
- 30% lower in vivo recovery of recFIX
Replacement strategies

- **Episodic (on-demand)**
  - Missing factor is replaced at onset of bleeding
    - 1 unit/kg FVIII = 2% increase in plasma FVIII activity
    - 1 unit/kg FIX = 1% increase in plasma FIX activity

- **Prophylactic**
  - Administered to prevent bleeding
  - Recommended by:
    - National Hemophilia Foundation
    - World Federation of Hemophilia

Prophylaxis

- **Primary prophylaxis**
  - Factor given to prevent complications

- **Secondary prophylaxis**
  - Factor given after onset of complications to prevent recurrence

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Prophylaxis versus Episodic Treatment to Prevent Joint Disease in Boys with Severe Hemophilia

Marsh J, Marco Johnson, M.D., Thomas C. Walter, M.D., Amy D. Staley, M.D., Bernie Bok, M.D., W. Blake, M.D., Michelle R. Marden, M.D., Ruth E. Uchida, M.D., J. David Negrin, M.D., Michelle J. Marco-Johnson, M.D., David J. Hurley, M.D., George G. Buchanan, M.D., Donna Gladstone, M.D., John M. Walshe, M.D., and John V. Flannery, M.D., Ph.D.
Main results

• At 6 years of age:
  – 93% of boys receiving prophylaxis had normal joints
  – 55% of boys receiving episodic infusions had normal joints
  – Relative risk of joint damage in boys receiving episodic infusions was 6.1
• High titer inhibitors detected in two boys receiving prophylactic infusions
• CNS hemorrhage occurred in three boys receiving episodic infusions

Bleeding episodes

Factor usage
Inhibitors

- Most serious treatment related complication
  - 15-50% in hemophilia A
  - 1-3% in hemophilia B (associated with anaphylaxis)
- Strongest determinant of risk of inhibitor development is mutation type
  - Large deletions
  - Inversions
  - Nonsense mutations
  - Splice site mutations
- No evidence for difference between inhibitor development in those exposed to plasma versus recombinant concentrate

Inhibitors and acute bleeding

- Activated prothrombin complex concentrates
  - Only FEIBA available in United States
  - Contains activated clotting factors, including FVIIa
  - Dosed at 75-100 units/kg every 12 hours
  - Most common side effect is thrombosis
- Recombinant activated factor VIIa
  - Dosed at 90-120 mcg/kg every 2 hours
  - Licensed for treatment of:
    - Hemophilia A or B with inhibitors
    - FVII deficiency
    - Glanzmann thrombasthenia (absence of gp IIb/IIIa)

Inhibitor reduction

- Immune tolerance reduction
- Immunomodulation
- Immunoadsorption and plasmapheresis
**Immune tolerance induction (ITI)**

- Repetitive doses of factor ± immunosuppressive therapy
- May have initial rise in antibody titer (anamnestic response)
- Immune tolerance must be maintained by continued exposure to infused factor
- Predictors of success of ITI
  - Inhibitor titer < 10 BU at start of ITI
  - Peak inhibitor titer < 200 BU
  - Presence of inhibitor < 2 years prior to start of ITI
  - Low risk mutations (small insertions/deletions, nonsense mutations)

**Immunomodulation**

- First reported in 1965, using mercaptopurine in conjunction with high dose factor replacement
- Agents reported
  - Corticosteroids
  - Cyclophosphamide
  - Azathiaprine
  - Vincristine
  - Cyclosporin
  - Tacrolimus
  - Mycophenolate mofetil
  - Rituximab
  - IVig

**Immunoadsorption/plasmapheresis**

- Extracorporeal strategies for removing pathological antibodies rapidly, though transiently
- Plasmapheresis replaces patients plasma with donor plasma, thereby reducing inhibitor titer
- Allows for use of FVIII or FIX concentrate for acute bleeding episodes or invasive procedures
- Immunoadsorption binds Fc portion of IgG to protein A
Adjuvant bleeding therapies

- Desmopressin
- Antifibrinolytic agents
  - ε-aminocaproic acid
  - Tranexamic acid
- Topical agents
  - Fibrin sealants
  - Floseal matrix
  - Topical thrombin

Desmopression

- For use in von Willebrand disease or mild/moderate hemophilia A
- Mechanism of action
  - Increased von Willebrand factor levels with resultant increased FVIII levels
  - Stimulation of platelet adhesion
  - Increased expression of tissue factor
- Dosing
  - Intravenous: 0.3 mcg/kg iv every 12 hours up to 3 doses
  - Intranasal: every 12 hours up to three doses
    - < 50 kg: one puff
    - ≥ 50 kg: two puffs

Antifibrinolytics

- Inhibit proteolytic activity of plasmin, therefore inhibiting fibrinolysis
- Indicated for mucosal bleeding:
  - Oral
  - Nasal
  - Menstrual
- Contraindicated in:
  - DIC
  - Thromboembolic disease
  - Upper urinary tract bleeding
Topical agents

• Fibrin sealants
  – Mixture of fibrinogen and thrombin concentrates
  – May contain FXIII or aprotinin
  – From human or bovine plasma
  – > 20% incidence of antibodies against thrombin
• FloSeal Matrix-bovine gelatin/human thrombin
• Thrombin-JMI-bovine thrombin
• BioGlue-bovine albumin/glutaraldehyde
• CoSeal-PEG polymer that cross-links proteins
• Quickclot-zeolite (dehydrates blood)

Future directions

• Factor repletion
• Genetic therapies
  – Gene therapy
  – Premature termination codon suppression

Future of factor repletion

• Main area of research has been prolonging half-life of factor concentrates
• Approaches explored to date:
  – Sustained delivery
  – Chemical modification of factor molecule
  – Genetic alteration
**Sustained delivery of FVIII**

- Release protein into blood stream more slowly, thereby increasing bioavailability
- Pegylated liposomal FVIII - Bayer
  - Prolonged mean number of days between bleeding episodes
  - No change in measured half-life
  - Pulled from phase 2 trial

**Chemically modified FVIII**

- Blocks receptor-mediated clearance
- Conjugates FVIII with hydrophilic polymer
  - Polyethylene glycol
  - Sialic acid
- Creates a molecular “shield” around FVIII molecule
- This strategy has been accomplished in many complex proteins
- Unknowns about this strategy
  - Clearance over an extended period of time
  - Alterations in immunologic properties

**Genetic alteration of FVIII molecule**

- Prevention of proteolysis
  - A2 subunit covalently cross-linked to A3 subunit
  - Activated protein C cleavage sites blocked
- Interference of FVIII clearance
  - Has not yet been accomplished
  - All attempts have resulted in decreased activity
- Generation of fusion proteins
  - FVIII molecule combined with either albumin or Fc fragment of IgG
  - Fc:FVIII fusion protein now in phase 3 trials (Biogen)
Gene therapy

- Goal is to replace dysfunctional gene with an exogenous functional gene
- Hemophilia is perfect condition for gene therapy
  - Relatively prevalent
  - Molecular pathway well studied
  - Caused by single gene mutations
  - Wide therapeutic window
  - FVIII and FIX do not need to be expressed by endothelial cells or hepatocytes
  - Monitoring of factor levels simple

Gene therapy

- Challenges
  - FVIII is a large molecule with a large gene making insertion into gene delivery systems difficult
  - Murine, canine, and non-human primate studies have not predicted the lack of efficacy and adverse events seen in human trials
  - Inhibitor development has been demonstrated

Premature termination codon suppression

- Nonsense mutations of either FVIII or FIX typically cause severe disease
- Caused by:
  - Base pair substitutions
  - Insertions
  - Deletions
  - Leading to the production of truncated proteins
Premature termination codon suppression

- PTC124 (Ataluren) oral agent that promotes a read-through of nonsense codons.
- Phase I trials have demonstrated the safety of PTC124 in healthy volunteers.
- Phase II trials have demonstrated efficacy of PTC124 in treatment of cystic fibrosis.
- Currently a phase II trial of 28-day treatment cycles of PTC124 in hemophilia is underway.
  - Theoretically decrease risk of inhibitor formation that complicates factor infusion and gene therapy.

Hemophilia research at OHSU

- Basic lab
  - Bone mineral deficiencies in FVIII deficient mice.
- Clinical trials
  - PTC124
  - Fc:FVIII fusion molecule
  - Long-acting FVIIIa
- Physical therapy protocols
  - Gait abnormalities in bleeding disorders.
  - Carbon fiber splints in bleeding disorders.